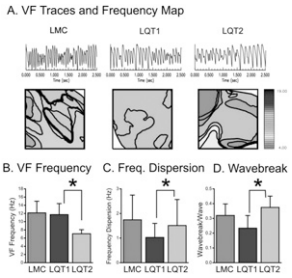
dynamics in transgenic rabbit models of LQT1, LQT2, and littermate controls (LMC) with optical mapping (n=6). VF frequency (VFF) analysis showed wave propagation to be slowest in LQT2 in line with APD gradient in groups (LQT2=7.06Hz, LQT1=11.72Hz, LMC=12.14Hz). Despite low frequency in LQT2, complexity of VFs was comparable to LMC. Further pattern analysis revealed that LQT2 exhibited higher incidence of wave breaks per wave while LQT1 shows even lower than LMC (LQT2=0.374 vs. LQT1=0.234, LMC=0.320). These results highlight role of I_{Kr} vs. I_{Ks} in VF maintenance, suggesting that VFs in LQT1 and 2 are maintained by different mechanisms such as wave breaks and re-initiation of reentry vs. triggered activity.



1511-Pos Board B403

Developmental Changes in Potassium Channel Expression in the Canine Heart: Implications for Sudden Infant Death Caused by Arrhythmias

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Background and Rationale: It is believed that at least 10% of Sudden Infant Death syndrome cases are due to cardiac arrhythmia, however, developmental changes in the electrophysiological characteristics are not well defined in the higher mammalian heart. The present study examines the contribution of the inwardly rectifying K+ current (IK1), the transient outward K+ current (Ito) and the delayed rectifier K+ currents (IKr and IKs) to repolarization in the canine neonate myocardium.

Methods and Results: Single cells were obtained from 2-3 week old canine neonate hearts subjected to enzymatic dispersion. Ventricular cells were isolated from both the left and right ventricles. Compared with adult ventricular cells, the size of neonate ventricular cells were approximately 6 times smaller. Action potential recordings showed a lack of phase 1 repolarization in neonatal myocytes. Accordingly, voltage clamp studies showed no Ito in neonatal myocytes. The inwardly rectifying potassium current IK1 was similar between neonate and adult. Measurement of the delayed rectifier(s) showed the presence of IKr, but not IKs. Additionally, mRNA levels for KCNQ1, KChIP2 and Kv4.3 were more than 2-fold lower in the neonatal heart compared with the adult heart;however, KCNH2 expression was unchanged.

Conclusion: Repolarization in 2-3 week old canine neonate ventricle is due mainly to the presence of IK1 and IKr. Neither IKs nor Ito were recorded at this stage of development. These findings point to a reduced repolarization reserve in ventricular myocytes isolated from canine neonatal hearts when compared to those of adults. Our data may help explain why infants are more vulnerable to developing QT prolongation and arrhythmias.

1512-Pos Board B404

Tuning L-Type Ca^{2+} Current Properties to Suppress Early After depolarizations

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Early afterdepolarizations (EADs) are highly arrhythmogenic transient depolarizations occurring during phase 2 or 3 of the cardiac action potential. We recently reported that EADs are highly sensitive to minimal shifts (by 1-5 mV) in the half activation/inactivation potentials ($V_{1/2}$) of the L-type Ca^{2+} current ($I_{Ca,L}$), such that $V_{1/2}$ modifications which reduce the window current voltage range were highly effective in suppressing EADs (Madhvani *et al.*, 2011). To better understand the underlying mechanisms, we have further explored the relevance of $I_{Ca,L}$ biophysical parameters to EADs formation. We took advantage of the dynamic clamp technique, a hybrid experimental-computational system permitting real-time introduction of a programmable conductance into a myocyte under current clamp. A consistent EAD regime was achieved by pacing ventricular myocytes at 0.2 Hz in the presence of 600 μM H_2O_2. The native $I_{Ca,L}$ was abolished with 20 μM nifedipine, and replaced with the dynamic clamp-generated $I_{Ca,L}$ to recapitulate EADs. We found that altering the slope of the steady-state activation curve had profound effects on EAD take-off potential and amplitude. For $z = 3.2\ e^0$, EAD amplitude was 22 ± 1.7 mV (take-off potential to peak). Steepening the voltage dependence of activation ($z = 5.7\ e^0$) increased the take-off potential and reduced EAD amplitude to 5.6 ± 0.6 mV. Steepening the voltage dependence of activation beyond $z = 5\ e^0$ resulted in small voltage oscillations (<1 mV) without frank EADs. In contrast, making the voltage dependence shallower increased EAD amplitude, especially when combined with a reduction of the non-inactivating component of $I_{Ca,L}$ (pedestal). Combined with our previous findings, our results show how multiple $I_{Ca,L}$ parameters affect EAD formation, providing a template for the development of therapeutic interventions.

1513-Pos Board B405

Hypotension in SM-Specific TRIC-A-Transgenic Mice

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TRIC (trimeric intracellular cation) channel subtypes, namely TRIC-A and TRIC-B, likely mediate counter-ion movements coupled with rapid Ca^{2+} release from intracellular stores in various cell types. The Tric-a-knockout mice suffered from hypertension due to enhanced myogenic tone in resistance arteries. Conversely, smooth muscle (SM)-specific Tric-a-transgenic mice developed hypotension. To maintain vascular tonus, two Ca^{2+} release mechanisms are functioning in vascular smooth muscle cells (VSMCs); incidental opening of ryanodine receptors (RyRs) generates local Ca^{2+} sparks to induce hyperpolarization, while agonist-induced activation of inositol trisphosphate receptors (IP3Rs) evokes global Ca^{2+} transients causing contraction.

To investigate the mechanism of hypotension in the Tric-a-transgenic mice, we performed Ca^{2+} imaging and Ca^{2+} spark measurements. Mutant VSMCs from the transgenic mice showed elevated Ca^{2+} spark generation, enhanced spontaneous transient outward currents (STOCs) and lowered resting Ca^{2+} levels.

The observation may suggest that overexpression of TRIC-A channels activates RyRs and enhances hyperpolarization signaling generated by functional coupling between RyRs and BK channels in VSMCs. In this situation, deactivated voltage-dependent Ca^{2+} channels may reduce resting Ca^{2+} levels and spontaneous tonus in the mutant VSMCs.

1514-Pos Board B406

A Novel Cav1.1-K1245Q Mutation Leading to Hypokalemic Periodic Paralysis

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Hypokalemic periodic paralysis (HypoPP) is the most common form of the periodic paralysis. It is caused by mutations in two voltage-gated ion channels of skeletal muscle, Cav1.1 (HypoPP-1) and Nav1.4 (HypoPP-2). Almost all HypoPP-causing mutations replace positive amino acids in the S4 voltage sensor of the channels. S4 mutations in Nav1.4 channels have already been shown to conduct sodium or protons through the omega pore which cause depolarization and impair the action potential generation. A leak current conducted by muscle fibers from HypoPP-1 patients was shown; however it was not formally demonstrated to be conducted by an omega pore. A novel mutation K1245Q in S4 of domain IV of Cav1.1 was identified in a patient who expressed weakness attacks with low serum potassium suggestive of HypoPP. These attacks have also been provoked by carbohydrate application. Further investigations revealed this mutation in other affected family members. To investigate possible gating alterations in the Cav1.1-K1245Q mutation, we performed whole-cell patch clamp measurements. A GLT cell line from muscular dysgenic mice (mdg) was cultured and transiently transfected with cDNA of wild type (WT) or mutant (K1245Q) Cav1.1. Calcium current amplitudes and voltage dependencies of activation of WT and K1245Q did not show significant differences in absolute or relative values. This data does not explain the patients pathogenesis. Omega currents produced by HypoPP Nav1.4 mutations might also exist in this Cav1.1-K1245Q mutant which could contribute to the phenotype of the patients. The corresponding measurements are currently performed, the results will be presented.

1515-Pos Board B407

The Chloride Channelopathy in Knockout Mice of Muscleblind-Like Proteins

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Myotonic dystrophy (DM) is the most prevalent human dystrophy; it results from a *spliceopathy* in which muscleblind-like (MBNL) regulatory proteins are sequestered by expanded CUG triplet repeats. In order to investigate the specific role that MBNL proteins play on the functional expression of chloride (ClC-1) channels, we studied the chloride currents (ICl) in fibers isolated from FDB muscles of adult knockout (KO) mice lacking MBNL1, MBNL3, or both (MBNL1/3 DKO). ICl were recorded in fibers voltage clamped with 2 microelectrodes, internally equilibrated with 70 mM intracellular chloride, and bathed in TEA-Cl solution. We found that ICl records in fibers from the three knockout strains display kinetic and voltage-dependent properties comparable to those in control fibers (129SV mice). However, the maximal peak ICl (peak-ICl_{max}), and the maximal conductance calculated from them (gCl_{max}), varied markedly among strains. Both peak-ICl_{max} and gCl_{max} are significantly smaller (~34%, $p<005$) in fibers of adult MBNL1 KO mice, than in those of the controls. The persistently impaired functional expression of ClC-1 channels contrasts with the transient chloride channelopathy of the HSA^{LR} model of DM. Furthermore, while ICl records in fibers of MBNL3 KO mice are identical to those from their control counterparts, peak-ICl_{max} in fibers of MBNL1/3 DKO mice show more severe reductions (~50%, $p<005$) than those of MBNL1 KO. These interesting results suggest novel synergistic regulatory interactions between MBNL proteins which ultimately affect the functional expression of ClC-1 channels. This work was supported by NIH grants AR047664, AR041802, and AR054816. Precursors of MBNL1 mice were kindly provided by Dr. M. Swanson, University of Florida.

1516-Pos Board B408

To the Molecular Mechanism of Mechanoelectrical Transduction in Cell

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Admittedly, mechanical deformation governs intracellular potential by means of specific stretch-activated channels in cell membrane. At the same time, the key mechanism of the mechanical stimulus (stretch) transduction in electrophysiological response is still not clear. Numerous studies by different authors have convincingly demonstrated the cytoskeleton sub-membrane structures (cortex) critical need for the mechanoelectrical transduction providing. From the physicochemical point of view, the cytoskeleton as a whole, and the cortex in particular resembles a polyelectrolyte hydrogel, i.e., a 3D biopolymer network with the electric charges localized on the macromolecular filaments, and with free counterions dispersed in the liquid phase inside the network. Presented investigation addresses the possible mechanism of stretch on cell electrochemical potential change, based on the physicochemical properties of cytoskeletal network. Synthetic polyelectrolyte gels were used as an experimental model of the cytoskeleton. We have found that axial deformation of polyelectrolyte gel shifts gel potential to depolarization. The decrease of potential with gel is the result of diminishing of counterion concentration inside the gel. The underlying mechanism of it is likely the universal process of counterion adsorption on charged polymer filaments due to the decrease of the distance between polymer filaments owing to gel elongation. Thus, the physicochemical properties of the gel network may affect the balance of ions between the cortex and liquid phase of the cell. Independently of the activity of stretch-activated channels, stretch of the cortex network is able to diminish the absolute value of cell potential. On the other hand, we may suppose also that such depolarization is the main factor that determines stretch-activated channels activity.

1517-Pos Board B409

Canonical WNT Pathway Enriches Cardiac Pacemaker Cell Population During Embryonic Stem Cell Differentiation

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Background: Embryonic stem cells (ESCs) can be guided to differentiate into cardiomyocytes by blocking canonical Wnt pathway (e.g., with Dkk-1) during cardiac specification stage. We hypothesized that canonical Wnt signaling may be an important negotiator during cardiac progenitors' commitment towards pacemaker or atrial/ventricular lineages.
Methods: Mouse ESCs were treated with activin-A/BMP-4 for 40 hours in a defined medium to initiate cardiac differentiation. Flk-1+/PdgfR-α+ cardiac progenitors are FACS-purified and seeded as monolayers with Dkk-1 (day-0).
Results: At day-4, ~65% of cells are positive for cTnT, a pan-cardiomyocyte marker. Some cTnT-positive cells express one or more of pacemaker-lineage markers, Shox2/Tbx18/Tbx3/Hcn4. Spontaneously-beating areas are observed starting day-2, and some single cells exhibit spontaneous, rhythmic action potentials with hallmark pacemaker electrophysiology such as phase-4 depolarization and depolarized maximal diastolic potential. Still, the monolayers beat in syncytium, resembling the passive contractions of atrial/ventricular myocardium. Removal of Dkk-1 significantly increases pacemaker gene transcript levels, Tbx18 and Shox2 by 5-fold ($p<0.05$, n=4), Hcn1 and Hcn4 by 2-fold ($p<0.05$, n=4) compared to the cells cultured with Dkk-1. Conversely, ventricular/atrial lineage markers, Nkx2.5 and Scn5a were suppressed by 4- and 8-fold, respectively, compared to control ($p<0.05$, n=4). In contrast to the syncytial contractions of the monolayers cultured with Dkk-1, intact canonical Wnt signaling (no Dkk-1) induces formation of discrete, node-like structures which beat autonomously. The beating rates of cells cultured without Dkk-1 are ~3x faster than that of cells cultured with Dkk-1 (161.5 ± 11.5 vs. 48.0 ± 2.9 bpm, $p<0.01$, n=4) at week-2. Single spontaneously-beating cells isolated from no-Dkk-1 group are frequently spindle-shaped replicating the morphology of genuine sinoatrial node pacemaker cells.
Conclusions: Endogenous, canonical Wnt pathway promotes differentiation of mouse cardiac progenitor cells into pacemaker cells rather than to normally-quiescent cardiomyocytes.

1518-Pos Board B410

Parametric Sensitivity Analysis of the Most Recent Computational Models of Rabbit Cardiac Pacemaking

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The cellular basis of cardiac pacemaking activity, and specifically the quantitative contributions of particular mechanisms, is still debated. Reliable computational models of sinoatrial node (SAN) cells may provide mechanistic insights, but competing models are built from different data sets and with different underlying assumptions. To understand quantitative differences between alternative models, we performed thorough parameter sensitivity analyses of the SAN models of Maltsev & Lakatta (2009) and Severi et al (2012). Model parameters were randomized to generate a population of cell models with different properties, simulations performed with each set of random parameters generated 14 quantitative outputs that characterized cellular activity, and regression methods were used to analyze the population behavior.
Our analysis pointed out that the two models, exhibit clearly different (sometime even opposite) sensitivity to several parameters. As relevant examples: (1) Na+-K+ pump activity, rapid delayed rectifier current (IKr) activation and SR Ca^{2+} pump activity had a greater effect on cycle length (CL) in the Maltsev model; (2) conversely, parameters describing the funny current (If) had a greater effect on CL in the Severi model; (3) changes in IKr conductance (GKr) had opposite effects on action potential (AP) amplitude in the two models.
Within the population, a greater percentage of model cells failed to exhibit APs in the Maltsev model (27%) compared with the Severi model (7%), implying greater robustness in the latter. Confirming this initial impression, bifurcation analyses indicated that smaller changes in GKr or Na^{+}-K^{+} pump activity led to failed APs in the Maltsev model.
Overall, the results suggest experimental tests that can distinguish between models and alternative hypotheses, and the analysis offers strategies for developing anti-arrhythmic pharmaceuticals by predicting their effect on the pacemaking activity.

1519-Pos Board B411

Mathematical Modelling of the Autonomous Activity of Cultured Neonatal Rat Ventricular Myocytes

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Problematic. The biological pacemaker is a new therapeutic approach that could lead to optimized treatment of bradycardia. A possibility is the development of a thin sheet of cardiomyocytes, cultured to obtain a target activation rate. Fundamental research, often conducted with neonatal rat ventricular myocytes (NRVMs), partially revealed two basic coupled mechanisms of automaticity termed Voltage Clock (synergy of membrane currents) and Calcium Clock (internal oscillations of calcium concentration). To date, no ionic model is able to reproduce in silico the autonomous activity found in cultured NRVMs. The present project aims to fill this gap.
Methods. A non-automatic NRVM ionic model (Korhonen-Tavi, 2009) is modified according to documented Voltage and Calcium Clocks mathematical formulations. The myocytes are cultured for 48 hours at low density, allowing cells to remain single on dishes. Autonomous action potentials (APs) are measured with patch clamp method, and calcium transients (CTs) with Fluo-4 AM